



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/82, 15/10, 9/12, 5/10, C12Q 1/68, A01H 5/00	A3	(11) International Publication Number: WO 00/08187 (43) International Publication Date: 17 February 2000 (17.02.00)
(21) International Application Number: PCT/EP99/05652 (22) International Filing Date: 4 August 1999 (04.08.99) (30) Priority Data: 98202634.6 4 August 1998 (04.08.98) EP (71) Applicant (for all designated States except US): VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR BIOTECH- NOLOGIE [BE/BE]; Rijvisschestraat 120, B-9052 Zwij- naarde (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): LEE, Jeong, Hee [KR/BE]; Spinnmolenplein 274 (22K), B-9000 Gent (BE). VERBRUGGEN, Nathalie [BE/BE]; Avenue des Saisons, 53, B-1050 Ixelles (BE). (74) Agent: DE CLERCQ, Ann; Ann De Clercq & Co. B.V.B.A., Brandstraat 100, B-9830 Sint-Martens-Latem (BE).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 29 June 2000 (29.06.00)
(54) Title: GENES INVOLVED IN TOLERANCE TO ENVIRONMENTAL STRESS		
(57) Abstract <p>The present invention relates to a method for obtaining polynucleic acids comprising coding sequences and/or genes involved in environmental stress resistance in plants, comprising the preparation of a cDNA library comprising coding sequences from siliques, introducing said coding sequences in yeast cells in a functional format and screening for polynucleic acids leading to an enhanced tolerance or resistance to environmental stress conditions in said transformed yeast cells. The present invention further relates to an isolated polynucleic acid obtainable by such a method as listed in Table 1 as well as recombinant polynucleic acid comprising the same. The present invention further relates to an isolated polypeptide encoded by a polynucleic acid of the invention. The present invention also relates to a method for producing a plant with enhanced tolerance or resistance to environmental stress, said method comprising introducing into a plant cell a recombinant DNA comprising a polynucleic acid as defined which when expressed in a plant cell enhances the tolerances or induces resistance to environmental stress conditions of said plant. The present invention particularly relates to plant cells, plants or harvestable parts or propagation material thereof transformed with a recombinant polynucleic acid as defined above.</p>		

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/05652

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639020 A	12-12-1996	US 5859337 A	12-01-1999
WO 9741152 A	06-11-1997	AU 3283197 A	19-11-1997
		EP 0907660 A	14-04-1999

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claim : 1 completely ; 2 partially

A method to identify cDNAs involved in environmental stress tolerance in plants by expressing a silique-specific cDNA library obtained from said plant in yeast cells and screening the yeast cells for enhanced environmental stress tolerance or resistance.

2. Claims: 5,6,15 completely , 2,3,4,11,12,13,14,
18-37 partially

An isolated cDNA as identified by SEQID 1 coding for a DBF2-related peptide characterized by SEQID 2; furthermore the use of the cDNA in methods to produce transgenic plants with enhanced environmental stress tolerance or resistance.

3. Claims: 10,17 completely , 2,3,4,11,12,13,14,
18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 5 coding for a c74-related peptide characterized by SEQID 6.

4. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 7 coding for a ADH2-related peptide characterized by SEQID 8.

5. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 9 coding for a catalase /catalase3-related peptide characterized by SEQID 10.

6. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 11 coding for a HSP90-related peptide characterized by SEQID 12.

7. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

by SEQID 13 coding for a peptide similar to a phosphoenolpyruvate carboxylase characterized by SEQID 14.

8. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 15 coding for a PR-protein characterized by SEQID 16.

9. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 17 coding for a protein similar to an ascorbate peroxidase characterized by SEQID 18.

10. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 19,21 coding for a protein similar to a phosphatase binding protein characterized by SEQID 20,22.

11. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 23,25 coding for a protein similar to a retinol dehydrogenase characterized by SEQID 24,26.

12. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 27,29 coding for a protein similar to a ribosomal protein characterized by SEQID 28,30.

13. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 31 coding for a protein similar to a protein transporter characterized by SEQID 32.

14. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 33 coding for a protein similar to a peptide transporter characterized by SEQID 34.

15. Claims: 2,3,4,11,12,13,14,18-37 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as invention 2; but limited to the cDNA as identified by SEQID 35 coding for an LCT1-related protein characterized by SEQID 36.

16. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 37 coding for an CYC1-related protein characterized by SEQID 38.

17. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 39 coding for an OSM1-related protein characterized by SEQID 40.

18. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 41 coding for an CUP1-related protein characterized by SEQID 42.

19. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 43 coding for an RAD7-related protein characterized by SEQID 44.

20. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 45 coding for an apocytochrome b-related protein characterized by SEQID 46.

21. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 47 coding for an LPPL1-related protein characterized by SEQID 48.

22. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 49 coding for a protein similar to an auxin binding protein characterized by SEQID 50.

23. Claims: 2,3,4,11,12,13,14,18-37 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as invention 2; but limited to the cDNA as identified by SEQID 51 coding for an CBP57-related protein characterized by SEQID 52.

24. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 53 coding for a calcineurin B-related protein characterized by SEQID 54.

25. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 55 coding for a calnexin-related protein characterized by SEQID 56.

26. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 57 coding for a calreticulin-related protein characterized by SEQID 58.

27. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 59,61 coding for a calmodulin-related protein characterized by SEQID 60,62.

28. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 63 coding for a DdMek1-related protein characterized by SEQID 64.

29. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 65 coding for an adenosine kinase-related protein characterized by SEQID 66.

30. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 67 coding for a human tyrosine kinase-related protein characterized by SEQID 68.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

31. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 69 coding for an ice-plant tyrosine kinase-related protein characterized by SEQID 70.

32. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 71 coding for a kinase C receptor-related protein characterized by SEQID 72.

33. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 73 coding for a HAT7-related protein characterized by SEQID 74.

34. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 75 coding for a RSEB-related protein characterized by SEQID 76.

35. Claims: 2,3,4,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 77 coding for a phosphatase 2C-related protein characterized by SEQID 78.

36. Claims: 2,3,4,11,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 121 potentially coding for a caltractin-related protein.

37. Claims: 7,8,16 completely; 2,3,11,13,14,18-37 partially

Idem as invention 1; but limited to the cDNA as identified by SEQID 3 coding for a HSP17.6-related protein characterized by SEQID 4.

38. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 79,81 coding for a LEA-related protein characterized by SEQID 80,82.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

39. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 83 coding for a protein similar to a PR-protein characterized by SEQID 84.

40. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 85 coding for a peroxidase-related protein characterized by SEQID 86.

41. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 87 coding for a ribosomal protein characterized by SEQID 88.

42. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 89 coding for a SAS1-related protein characterized by SEQID 90.

43. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 91 coding for a AIG2-related protein characterized by SEQID 92.

44. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 93 coding for a MTlc-related protein characterized by SEQID 94.

45. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 95 coding for a IPP2-related protein characterized by SEQID 96.

46. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 97 coding for a chlorophyll a/b binding protein characterized by SEQID 98.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

47. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention ; but limited to the cDNA as identified by SEQID 99 coding for glutathione transferase characterized by SEQID 100.

48. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 101 coding for kin1-related protein characterized by SEQID 102.

49. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 103 coding for Atmpk1-related protein characterized by SEQID 104.

50. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 105 coding for H2A-related protein characterized by SEQID 106.

51. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 107 coding for a protein with unknown function as characterized by SEQID 108.

52. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 109 coding for a protein with unknown function as characterized by SEQID 110.

53. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 111 coding for a protein with unknown function as characterized by SEQID 112.

54. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 113 coding for a protein with unknown function as

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

characterized by SEQID 114.

55. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 115 coding for a protein with unknown function as characterized by SEQID 116.

56. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 117 coding for a protein with unknown function as characterized by SEQID 118.

57. Claims: 2,3,9,11,12,13,14,18-37 partially

Idem as invention 2; but limited to the cDNA as identified by SEQID 119 coding for a protein with unknown function as characterized by SEQID 120.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/05652

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C12N15/10 C12N9/12 C12N5/10 C12Q1/68
A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PRÄNDL, R., ET AL. : "HSF3, a new heat shock factor from Arabidopsis thaliana, derepresses the heat shock response and confers thermotolerance when overexpressed in transgenic plants" MOLECULAR AND GENERAL GENETICS, vol. 258, May 1998 (1998-05), pages 269-278, XP002135096 the whole document --- -/--	2,3,7,8, 11-14, 16,18, 21, 24-26, 28-37



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

10 April 2000

Date of mailing of the international search report

27.04.00

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

Int .tional Application No

PCT/EP 99/05652

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KUSHNIR, S., ET AL.: "characterization of Arabidopsis thaliana cDNAs that render yeasts tolerant toward the thiol-oxidizing drug diamide" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 92, November 1995 (1995-11), pages 10580-10584, XP002127804 see also last paragraph the whole document ---	1,2
Y	WO 96 39020 A (UNIV CALIFORNIA) 12 December 1996 (1996-12-12) the whole document ---	1,2
Y	QUINTERO, F.J., ET AL.: "the SAL1 gene of Arabidopsis, encoding an enzyme with 3' (2'), 5'-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase activities, Increases salt tolerance in yeast" THE PLANT CELL, vol. 8, March 1996 (1996-03), pages 529-537, XP002092755 see last paragraph the whole document ---	1,2
Y	BABIYCHUK, E., ET AL.: "Arabidopsis thaliana NADPH oxidoreductase homologs confer tolerance of yeasts towards the thiol-oxidizing drug Diamide" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 44, 1995, pages 26224-26231, XP002127805 see last paragraph the whole document ---	1,2
Y	GIRAUDAT J ET AL: "ISOLATION OF THE ARABIDOPSIS AB13 GENE BY POSITIONAL CLONING" PLANT CELL, US, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 4, 1 October 1992 (1992-10-01), pages 1251-1261, XP002063682 ISSN: 1040-4651 the whole document ---	1,2
Y	WO 97 41152 A (UNIV NEW YORK) 6 November 1997 (1997-11-06) page 59, line 1 - line 5 ---	1,2

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/05652

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NAMBARA, E., ET AL.: "a mutant of Arabidopsis which is defective in seed development and storage protein accumulation is a new <i>abi3</i> allele" THE PLANT JOURNAL, vol. 2, no. 4, 1992, pages 435-441, XP002129247 the whole document ---	1,2
A	HELM, K.W. AND VIERLING, E.: "an Arabidopsis thaliana cDNA clone encoding a low molecular weight heat shock protein" NUCLEIC ACID RESEARCH, vol. 17, no. 19, 1989, page 7995 XP002129295 the whole document ---	1,2
A	YANG H ET AL: "Arabidopsis thaliana ECP63 encoding a LEA protein is located in chromosome 4" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, GB, ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 184, no. 1, 3 January 1997 (1997-01-03), pages 83-88, XP004093225 ISSN: 0378-1119 the whole document ---	1,2
A	MIZOGUSHI, T., ET AL.: "characterization of two cDNAs that encode MAP kinase homologues in Arabidopsis thaliana and analysis of the possible role of auxin in activating such kinase activities in cultured cells" THE PLANT JOURNAL, vol. 5, no. 1, 1994, pages 111-122, XP002129296 the whole document ---	1,2
P, X	LEE, J.H., ET AL.: "a highly conserved kinase is an essential component for stress tolerance in yeast and plant cells" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 96, May 1999 (1999-05), pages 5873-5877, XP002127807 the whole document -----	1,2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/05652

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1,2-4,7,8,10-14,16-37 (inventions 1,3,37)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.